

(a) culturing an identified species, or serogroup of a species of bacteria, to a desired size and harvesting therefrom cells of that species; or serogroup of a species of bacteria, as a wet cell pellet;

(b) obtaining from the wet cell pellet an essentially protein-free carbohydrate antigen by a series of substeps which comprise

(i). suspending the wet cell pellet from step (a) in an alkaline solution and mixing;

(ii). adjusting the pH to an acid pH with a strong acid;

(iii). separating the mixture from substep (b) (ii) into two layers;

(iv). removing the upper layer and adjusting its pH to approximate neutrality;

(v). adding to the product from substep (iv) a broad spectrum protease enzyme and digesting to destroy residual proteins;

(vi). adjusting the pH of the product from substep (b) (v) to an alkaline pH with a weakly alkaline aqueous solution and;

(vii). separating out an essentially protein free carbohydrate antigen of said species or serogroup of a species of bacteria;

(c) coupling to a chromatographic affinity gel through a spacer molecule the essentially protein-free carbohydrate antigen obtained in step (b);

(d) passing antibodies to the same species, or serogroup of a species of bacteria, as that cultured in step (a) or an Ig G cut thereof over the chromatographic affinity gel of step (c) to produce purified antigen-specific antibodies; and

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enzyme specific

carbohydrate

Legitim 04/137, 720 - 140-140 ng.
5 pm 04/137, 110 c - poly saccharide wall

(e) conducting an assay upon a liquid sample suspected of containing the same species, or serogroup of a species of bacteria, as that cultured in step (a), which assay comprises the step of detecting the crude carbohydrate antigen of said species or serogroup of a species of bacteria which is counterpart to the purified antigen of step (b), by contact of the liquid sample with a detection agent which essentially comprises labelled purified antigen-specific antibodies from step (d) hereof.

*Unbroken
clumping
Assay*

23 The method of claim 22 in which the species, or serogroup of a species of bacteria, in step (a) are Gram negative bacteria and the crude antigen component thereof sought to be detected in step (e) is a lipopolysaccharide.

24 The method of claim 22 in which the species or serogroup of a species of bacteria are Gram positive bacteria and the crude antigen component thereof sought to be detected in step (e) is a lipoteichoic acid, a teichoic acid, or a derivative of either.

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25 The method of claim 22 in which the species or serogroup of a species of bacteria are either Gram negative or Gram positive bacteria and the crude antigen component thereof sought to be detected is a capsular polysaccharide antigen.

26 The method of claim 22 in which the spacer molecule of step (c) is a protein molecule.

27 The method of claim 22 wherein the liquid sample of step (e) is water.

28 The method of claim 22 wherein the liquid sample of step (e) is a natural fluid of mammalian origin.

29 The method of claim 28 wherein the liquid sample of step (e) is human urine.

30 The method of claim 28 wherein the liquid sample of step (e) is obtained from a patient exhibiting clinical signs of a disease known to be caused by the bacteria cultured in step (a).

31 The method of claim 22 in which step (e) is an immunoassay process.

32 The method of claim 31 in which step (e) is an immunochromatographic ("ICT") immunoassay process.

33 The method of claim 32 in which the bacteria cultured in step (a) are *Haemophilus influenzae* type b bacteria and the crude antigen sought to be detected in step (e) is the capsular carbohydrate antigen of those bacteria.

34 The method of claim 22 in which step (e) is conducted by

(A) contacting a liquid sample suspected of containing the species, or serogroup of a species of bacteria cultured in step (a) of claim 22, or a crude carbohydrate antigen thereof, with an ICT device comprising a strip of bibulous material, which strip has

- (i) a first zone in which has been deposited a movable conjugate of a labelling agent and purified antigen-specific antibodies obtained in step (d) of claim 22, said labelling agent being selected from among those known to display a visible color change upon the formation of a labelled antibody-antigen-fixed antibody reaction product and
- (ii) a second zone having immovably bound thereto unconjugated purified antigen-specific antibodies obtained in step (d) of claim 22, which zone is equipped with a window for viewing color changes,

(B) allowing said liquid sample to flow laterally along said test strip to said first zone, where it picks up the deposited conjugate of label and purified antigen-specific antibodies;

(C) allowing said liquid sample and said conjugate of antigen-specific antibodies and label to flow together laterally along said test strip to said second zone, and

(D) within approximately 15 minutes after contacting the liquid sample with the test strip, observing through the aforementioned window whether a line of color indicating the presence in the sample of the suspected bacteria species, or serogroup of that species, has formed.

35 The method of claim 34 in which the bacteria are Gram positive bacteria and the crude antigen sought to be detected is a lipopolysaccharide antigen.

36 The method of claim 34 in which the bacteria are Gram negative bacteria and the crude antigen sought to be detected is a lipoteichoic acid, a teichoic acid or an ester of either.

37 The method of 34 in which the bacteria are Gram positive or Gram negative bacteria and the crude antigen sought to be detected is a capsular carbohydrate antigen.

38 The method of claim 34 in which the liquid sample is a natural fluid of mammalian origin.

39 The method of claim 38 in which the liquid sample is human urine.

40 The method of claim 39 in which the liquid sample is obtained from a patient exhibiting symptoms of a disease known to be caused by the species, or serogroup of a species of bacteria that was cultured in step (a) of claim 22.

41 The method of claim 34 in which the labelling agent is finely divided colloidal gold.

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~~434~~ The method of claim 39 in which the crude antigen sought to be detected is the capsular carbohydrate antigen of *Haemophilus influenzae* type b.

43 An ICT device for the detection of a carbohydrate antigen characteristic of a species or serogroup of a species of bacteria, which comprises a strip of bibulous material having

(a) a first zone in which has been movably deposited a conjugate of a labelling agent and purified antibodies specific to the crude carbohydrate antigen of the bacteria species, or serogroup of a species, suspected of being present in the liquid sample, and

(b) a second zone having immovably bound thereto a portion of unconjugated, purified antibodies specific to the same crude carbohydrate antigen, which zone is equipped with a window for viewing color changes; which device is further characterized in that antigen-specificity of the antibodies present in both zones has been attained by passing antibodies to the bacteria species or serogroup of a species of which the crude carbohydrate antigen is characteristic over a chromatographic affinity column to which is coupled a spacer molecule conjugated to an essentially protein-free carbohydrate antigen, which essentially protein-free carbohydrate antigen was obtained from a culture of the *Legionella* bacteria species, or serogroup of a species of bacteria of which the crude carbohydrate antigen is characteristic according to the following method:

- (i) harvesting cells from the culture in the form of a wet cell pellet;
- (ii) suspending the wet cell pellet in an alkaline solution and mixing;
- (iii) adjusting the pH of the resultant mixture to an acid pH with a strong acid;
- (iv) separating the acidified product from step (iii) into two layers;

- (v) removing the upper layer and adjusting its pH to approximate neutrality;
- (vi) adding to the product from step (v) a broad spectrum protease enzyme and digesting to destroy residual proteins;
- (vii) adjusting the pH of the product from step (vi) to an alkaline pH with a weakly alkaline aqueous solution; and
- (viii) separating out an essentially protein-free carbohydrate antigen.

44 The ICT device of claim 43 wherein the species or serogroup of a species of bacteria are Gram positive bacteria and the crude antigen to be detected is a lipopolysaccharide antigen.

45 The ICT device of claim 43 wherein the species or serogroup of a species of bacteria are Gram negative bacteria and the crude antigen to be detected is a lipoteichoic acid, a teichoic acid or an ester of either.

46 The ICT device of claim 43 wherein the species or serogroup of a species of bacteria are Gram negative or Gram positive bacteria and the crude antigen to be detected is a capsular polysaccharide antigen.

47 The ICT device of claim 43 wherein the labelling agent is finely divided colloidal gold.

48 A method for detecting a crude carbohydrate antigen characteristic of a bacteria species, or serogroup of a species, in a liquid sample which comprises the steps of

(a) contacting said liquid sample with the strip of bibulous material of the ICT device of claim 43;

(b) allowing said liquid sample to flow laterally along said test strip to the first zone of said device where it picks up a movable deposit of a conjugate of labelling agent and purified antibodies;

(c) allowing said liquid sample and said conjugate to flow together laterally along said test strip to the second zone of said device; and

(d) within approximately 15 minutes after contacting the liquid sample with the test strip, observing through the view window whether a line of color has appeared, indicating the presence in the test sample of the species, or serogroup of a species of bacteria, containing the crude carbohydrate antigen to which the purified antibodies are specific.

✓ 49 The method of claim 48 wherein the liquid sample is a natural fluid of mammalian origin.

50 The method of claim 49 wherein the liquid sample is obtained from a human patient exhibiting symptoms of a disease known to be caused by the bacteria species or serogroup of a species of which the crude antigen to be detected is characteristic.

✓ 51 The method of claim 50 wherein the liquid sample is human urine.

52 An ICT device according to Claim 43 in which the antibodies are present in a concentration of between 7.7 nanograms per square millimeter and 385 nanograms/square millimeter of surface area in both the first and second zones.

By this amendment, non-elected claims 1, 2 and 12-14 are retained herein pending the possible filing of a divisional application.